Jpn. J. Cancer Res. 86, 1179–1181, December 1995

N-Acetyltransferase Activity in the Urine in Japanese Subjects: Comparison in Healthy Persons and Bladder Cancer Patients

Sumiko Ishizu,[1] Chise Hashida,[2] Tomoyuki Hanaoka,[1] Keiko Maeda[3] and Yukihiko Ohishi[4]

[1]The Institute for Science of Labour, 2-8-14 Sugao, Miyamae-ku, Kawasaki 216, [2]Health Center, Yokohama National University, 156 Tokiwadai, Hodogaya-ku, Yokohama 240, [3]Department of Urology, Tokyo Women's Medical College, 1-8 Kawadacho, Shinjuku-ku, Tokyo 162 and [4]Department of Urology, Tokyo Jikeikai Medical College, 3-25-8 Nishishinbashi, Minato-ku, Tokyo 105

The activity of urinary N-acetylamino-transferase was determined by high-performance liquid chromatographic assay of acetylisoniazid and isoniazid after administration of isoniazid to healthy Japanese male and bladder cancer patients in Japan. The healthy subjects were 47 college students and 44 company employees ranging from 18 to 64 years old (mean±SD=34.5±13.7). The bladder cancer group consisted of 58 male and 13 female patients, ranging from 28 to 82 years old (mean±SD =60.8±11.6), who were being treated at several hospitals. The slow phenotype, defined as an acetylation ratio (acetylisoniazid/isoniazid) of less than 2.0, was observed in 13 (14.3%) of the 91 healthy subjects, and in 20 (28.2%) of the 71 bladder cancer patients; the difference between the two groups is significant ($P<0.05$). A histogram of the acetylation ratio values showed an overall leftward shift of the patient group, indicating low values of acetylation ratio in this group as a whole ($P<0.01$).

Key words: N-Acetyltransferase phenotype — Japanese healthy men — Bladder cancer — AcINH/INH ratio

N-Acetyltransferase (NAT), which metabolizes arylamines, has two phenotypes, slow and rapid. The activity of this enzyme has a major influence on the metabolism of aromatic amines, which play a role in carcinogenesis.[1] The NAT activity shows racial variation, suggesting the importance of genetic factors.[2–4] Sunahara *et al.*[5,6] studied the NAT phenotype in a large number of Japanese subjects and concluded that occurrence of the slow type was quite rare, amounting to only 11.5% (205 out of the total 1808 cases). Several studies[7–9] have indicated the slow acetylator phenotype is associated with increased risk of bladder cancer. However, Sone *et al.*[10] and Horai *et al.*[11] found no correlation between slow acetylator phenotype and occurrence of bladder cancer in Japan. According to our observations on occurrence of bladder cancer among factory workers exposed to aromatic amines, neither quantity nor period of exposure significantly influenced the rate of occurrence. We, therefore, assumed that acetylation may play an important role in carcinogenesis.

In this study, we estimated the ratio of slow acetylator phenotype among Japanese bladder cancer patients and healthy volunteers and examined the correlation between slow acetylation phenotype and bladder cancer.

SUBJECTS AND METHODS

The subjects consisted of 91 healthy Japanese males (47 college students and 44 company employees), whose age ranged from 18 to 64 (mean±SD=34.5±13.7) years, and 71 bladder cancer patients receiving treatment at hospitals in Tokyo, consisting of 58 males and 13 females, whose age ranged from 29 to 82 (mean±SD= 60.8±11.6) years. They were asked to answer questionnaires covering their past occupational history, in particular, exposure to specific chemicals and smoking and drinking habits. Written informed consent was obtained from each subject and confirmed orally prior to testing.

Urine samples were collected at 4 h after isoniazid (INH) administration (200 mg) and stored at −20°C until required. The INH and acetylisoniazid (AcINH) levels in the urine samples were measured by a method developed by Svensson *et al.*[12] and modified by Hayashi *et al.*[13] using high-performance liquid chromatography.

The NAT activity was evaluated in terms of the ratio of AcINH to INH and the NAT phenotype was defined as slow type for an acetylation ratio below 2.0 and rapid type for 2.0 and above. The proportions of the slow type in the two groups were calculated and the significance of the difference was evaluated statistically by means of the chi-square method and the median test.

RESULTS

The sex, age and smoking history of 91 healthy subjects and 71 bladder cancer patients in relation to slow phenotype are shown in Table I. There was little correlation between NAT activity and age (correlation coefficient=−0.013) in the healthy group. The patient group was predominantly male (58/71=81.7%), with a consid-

Table I. Various Characteristics of 91 Healthy Subjects and 71 Bladder Cancer Patients in Relation to Slow Acetylation Phenotype

	Healthy control subjects		Bladder cancer patients	
	All No. (%)	Slow type No. (%)	All No. (%)	Slow type No. (%)
	91	13 (14.3)	71	20 (28.2)
Sex				
Male	91/91 (100)	13/91 (14.3)	58/71 (81.7)	17/58 (30.4)
Female	0	0	13/71 (18.3)	3/13 (20.0)
Age (years)				
≦50	79/91 (86.5)	11/79 (13.9)	11/71 (15.5)	7/11 (63.6)
>50	12/91 (13.2)	2/12 (16.7)	46/71 (64.8)	10/46 (26.7)
Unknown[a)]			14/71 (19.7)	3/14 (21.4)
Smoking history				
Nonsmoker	45/91 (49.5)	7/45 (15.6)	18/71 (25.4)	7/18 (38.9)
<20 cigarettes/day	31/91 (34.0)	6/31 (19.4)	3/71 (4.2)	0
≧20 cigarettes/day	15/91 (16.5)	0	26/71 (36.6)	9/26 (34.5)
Unknown[a)]			24/71 (33.8)	4/24 (16.7)

a) No history available on patients.

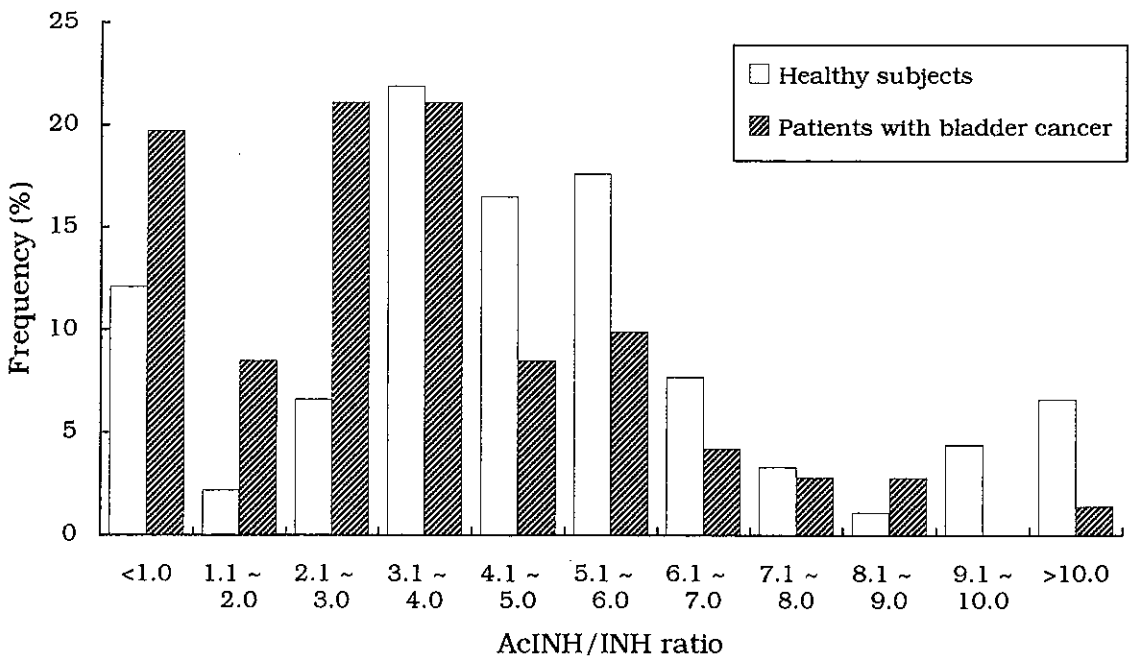

Fig. 1. Distribution of values of acetylation (AcINH/INH) ratio in 91 healthy subjects and in 71 bladder cancer patients.

erable number over 50 years old. It is interesting that the proportion of slow type is higher below 50 years old than above 50 years old.

Fig. 1 shows a histogram of the NAT activity data. The numbers with slow phenotype are 13 (14.3%) out of 91 in the healthy group with a 95% confidence interval of 7.1% to 21.4% and 20 (28.2%) out of 71 in the patient group, with a 95% confidence interval of 17.7% to 38.6%. The proportion of the slow phenotype was obviously higher in the patient group than the control ($P<0.05$). The result of the median test is summarized in Table II; the number of patients having activity below the median, 48 people, is greater than the expected number, 35.5, and the difference is statistically significant ($P<0.01$).

Table II. Analysis of Acetylator Status of 71 Bladder Cancer Patients

	No. expected	No. found
Bladder cancer patients slower acetylator than median	35.5 (50%)	48 (67.6%)
Bladder cancer patients faster acetylator than median	35.5 (50%)	23 (32.4%)

DISCUSSION

Occurrence of the slow acetylator phenotype among healthy Japanese was reported to be infrequent, 11.4% (207 out of 1808 cases) by Sunahara *et al.*[5,6] using INH and 6.9% (13 out of 189 cases) by Horai *et al.*[14] using dapsone. Our value of 14.3% (13 out of 91 healthy subjects) obtained using INH is within the same range. There seems to be a consensus that the slow acetylator phenotype is closely associated with occurrence of bladder cancer.[1,8,9,15–17] However, studies on Japanese subjects by Sone *et al.*[10] and Horai *et al.*[11] found no significant difference in the proportion of slow type between the control and bladder cancer groups. Nevertheless, in the present study on Japanese subjects, we found a significant difference in the proportion of the slow phenotype between the healthy control and the bladder cancer groups. It is well known that NAT activity is little influenced by age and smoking habit.[7,18] Our study also confirmed this, with correlation coefficients of only 0.013 and 0.084 for the healthy group. Our observations of a higher proportion of slow phenotype for the age group of < 50 years old of the patients ($11/23 = 47.8\%$) and the overall lower values of NAT activities among the patients compared with the control suggest that NAT could have an important role in bladder carcinogenesis. Further examination of N-acetylator phenotypes among existing bladder cancer patients and workers occupationally exposed to aromatic amines in Japan seems justified. If genetic susceptibility to carcinogenesis is confirmed, it might be possible to develop prophylactic screening.

(Received April 28, 1995/Accepted September 8, 1995)

REFERENCES

1) Lower, G. M., Jr., Nilsson, T., Nelson, C. E., Wolf, H., Gamsky, T. E. and Bryan, T. E. N-Acetyltransferase phenotype and risk in urinary bladder cancer: approaches in molecular epidemiology. *Environ. Health Perspect.*, **20**, 71–79 (1979).
2) Evans, D. P., Manley, K. A. and McKusic, V. A. Genetic control of isoniazid metabolism in man. *Br. Med. J.*, **13**, 485–491 (1960).
3) Klark, D. W. J. Genetically determined variability in acetylation and oxidation — therapeutic implications. *Drugs*, **29**, 342–375 (1985).
4) Meyer, U. A. Genetic polymorphism of drugs metabolism. *Fundam. Clin. Pharmacol.*, **4**, 595–615 (1990).
5) Sunahara, S. Clinical findings of INH. *Bull. Jpn. Soc. Tuberc.*, **36**, 521–527 (1961) (in Japanese).
6) Sunahara, S., Urano, M. and Ogawa, M. Genetic and geographic studies on isoniazid inactivation. *Science*, **134**, 1530 (1961).
7) Evans, D. A. P., Eze, L. C. and Whisbley, J. The association of the slow acetylator phenotype with bladder cancer. *J. Med. Genet.*, **20**, 330–333 (1983).
8) Cartwright, R. A., Glashan, R. W., Rogers, H. A., Ahmad, R. A., Barham-Hall, D., Higgins, E. and Kahn, M. A. Role of N-acetyltransferase phenotypes in bladder carcinogenesis: a pharmacogenetic epidemiological approach to bladder cancer. *Lancet*, **ii**, 842–845 (1982).
9) Mommsen, S. and Aagaad, J. Susceptibility in urinary bladder cancer: acetyltransferase phenotypes and related risk factors. *Cancer Lett.*, **32**, 199–205 (1986).
10) Sone, S. Determination of N-acetyltransferase phenotype in urothelial cancer patients and healthy controls. *Acta Urol.*, **32**, 1085–1092 (1986).
11) Horai, Y., Fujita, K. and Ishizaki, T. Genetically determined N-acetylation and oxidation capacities in Japanese patients with non-occupational urinary bladder cancer. *Eur. J. Clin. Pharmacol.*, **37**, 581–587 (1989).
12) Svensson, J.-O., Muchtar, A. and Erisson, O. Ion-pair high performance liquid chromatographic determination of isoniazid and acetylisoniazid in plasma and urine. *J. Chromatogr.*, **341**, 193–197 (1985).
13) Hayashi, O., Hashida, C. and Ishizu, S. Determination of AcINH/INH ratio by HPLC. *Jpn. J. Hyg.*, **47**, 295 (1992).
14) Horai, Y. and Ishizaki, T. N-Acetylation polymorphisms of dapsone in a Japanese population. *Br. J. Clin. Pharmcol.*, **25**, 487–494 (1988).
15) Miller, M. E. and Cosgriff, J. M. Acetylator phenotype in human bladder cancer. *J. Urol.*, **130**, 65–66 (1983).
16) Motanosky, G. M. and Elliot, E. A. Bladder cancer epidemiology. *Epidemiol. Rev.*, **3**, 203–229 (1981).
17) Hein, D. W., Rustan, T. D., Doll, M. A., Bucher, K. D., Ferguson, R. A., Feng, Y., Furman, E. J. and Gray, K. Acetyltransferase and susceptibility to chemicals. *Toxicol. Lett.*, **64/65**, 123–130 (1992).
18) Philip, P. A., Gayed, S. L., Rogers, H. J. and Crome, P. Influence of age, sex and body weight on the dapsone acetylation phenotype. *Br. J. Clin. Pharmacol.*, **23**, 709–713 (1987).